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PALAEOBOTANY AND THE ORIGIN OF THE ANGIOSPERMS

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The origin of flowering plants is still one of the greatest problems of evolution and has a direct bearing on the taxonomy of the group. The questions at issue concern not only the identification of the more primitive families but the morphological nature and origin of the flower, and they may lead us to ask how and why the classical concepts of floral morphology have originated. The problem has been hitherto studied almost entirely by the comparison of forms now living, and though a vast amount has been written no certain or agreed conclusions have been reached. Is it possible that palaeobotany can make a real contribution to the discussion? We can never hope to find the remains of complete lineages of ancient plants; the conditions under which their preservation could have occurred were too rare. But we do find examples of vegetation taken in a random way over a period of 300–400 million years, and these ought to give us some general idea of the evolutionary trends in plant structure over the period. Our samples are mainly the plants of low-lying areas near deltas and estuaries, and their probable relation to their contemporary forms should be assessed with this in mind.

At the outset we are faced with the question of the characters which distinguish angiosperms from other plants. Assuming that gradual morphological change has taken place during the evolution of the group, how are we to distinguish a primitive flowering plant if we find one? The possession of a closed ovary is not enough, for in the past plants which were far removed from the main assemblage of modern flowering plants, existed (68) with this peculiarity. Leaf form certainly underwent great change. Stem structure is not decisive. The most reliable single character is the

possession of flowers. But we must then decide what we mean by a flower. Almost all botanists are agreed that the flower is "a specialized strobilus" (or a bud) "in which the lower sporophylls have become sterilized to form sepals and petals and the upper have changed into stamens and carpels" (66). The axis of the strobilus is thought to have shortened to form the receptacle in hypogynous flowers of the primitive type, while in the more specialized types it has become depressed into a cup containing the carpels. Few modern writers specify exactly what they mean by a sporophyll, but the term implies a foliar structure or a modified leaf. Inquiry into the origin and development of the concept shows that it originated with Goethe in pre-evolutionary days as a subjective generalization (71) for which objective evidence could be neither expected nor required. This generalization was taken up by Darwin and used as an argument for evolution, since when it has been regarded as too axiomatic to need demonstration. Consequently, what was a mental picture has assumed a historical significance, and no serious attempt has been made to verify or disprove it. Now if this classical view of floral morphology, with its implications as to the course of floral evolution, is true, it should receive support from historical evidence of the rocks. We have large samples of undoubted flowering plants preserved at frequent intervals over some 90 millions of years, and these ought to provide evidence of the progress from hypogynous to peri- and epigynous flowers and of a progression from sporophylls of a more leaf-like to less leaf-like types. Is there such evidence?

FOSSIL PLANTS AS TESTS OF CURRENT THEORIES

In endeavoring to trace the history of our flowering plants backwards we may notice that our samples from Tertiary and Upper Cretaceous rocks (see Appendix) are numerous (100–400 species) and come from different parts of the world; the majority, however, can be assigned to living families. A number of Pliocene and more recent fossils can be identified with living species, but while the generic identification of Miocene angiosperms is clear, many of the species are no longer referable to living forms. Of the Eocene species identified by fruits and seeds, only some 25 per cent of the floras can be referred to living genera, but in their leaves, seeds and fruits most of the remainder are so close to living genera that their

general affinities cannot be questioned. Thus, while there has been morphological change during the last 60 million years or more, it seems to have been very slow in the many plants which occur in our records.

Before we can attempt to assess the direction of the changes we must be sure of our identifications. Up to the present, the angiosperms in Tertiary and Cretaceous floras have been identified mainly from leaf impressions in fine grained rock. Hooker long ago (34) cast doubts upon the specific identification of fossil plants, and many authors have since felt that leaf remains had too often been identified from evidence which no botanist would accept in work on modern plants. But when the leaves are found as really well preserved or mummified specimens, and comparison is made with a wide range of herbarium material (17), significant determinations may be reached. Recent study of cuticular characters has added many additional features for comparison and has led to the recognition of several genera not previously known from Tertiary rocks (4, 5, 6, 7, 31, 32, 65). The value of cuticle characters has been questioned (50) but a critical survey of the whole field by Edwards (20) shows their undoubted importance. The collection and identification of fruits and seeds which are abundant in certain fresh-water deposits confirms much of the evidence from leaves and adds information which is of the greatest significance.

In this field of study the Pliocene beds have provided rich seed floras (51).[1] Miocene seeds and fruits are known from several European localities (82, 39). Many forms are known from the Oligocene (39, 52), while most important work has been done on the Eocene floras (16, 53).[1] The extraction and identification of seeds from fresh-water rocks in North America has been commenced (21), but it seems certain that very much remains to be done.

A distinct and new line of progress has come with the collection and study of pollen from shales and other rocks where it is often abundant. Wodehouse (80, 81) has identified more than 100 species among a large number of distinct forms of pollen grains from the Eocene Green River formation, and a commencement of this very promising work has been made in Europe. The number of well preserved remains of flowers is small but not inconsiderable

[1] The references given comprise only a small selection from the literature.

specimens, like the well-known flowers in amber (17A), are of importance as showing how little has been the change in floral structure over the many millions of years since Oligocene times.

One of the oldest sources of evidence about the Tertiary dicotyledons is derived from the study of petrified woods, of which a large variety has been found in different strata and areas. The majority were studied and named a long time ago when our knowledge of wood anatomy was far from complete; their suggested affinities, consequently, are very doubtful. Even at the present time authorities are uncertain of the extent to which genera and even families can be recognized by the structure of their secondary wood, but there can be little doubt that the recent activity in this branch of study will be of great value to palaeobotany. A valuable prelude to a reconsideration of the whole field has been the production of a catalogue of all the described species (19), and critical studies of certain groups have been undertaken (2, 3). All we can now say is that between 40 and 50 families appear to be represented by the Tertiary woods so far described.

Combining the evidence from all the different lines of work we can now be quite sure of the general succession of plant forms on the shores of the estuaries in North Temperate regions throughout the Tertiary period, in terms of the existing flora of the world, and that a comparatively small percentage of the forms discovered are totally unlike any living species. Some of our recent progress has been summarized by Hirmer (30) who gives lists of the species found. With these before us it should be possible to gauge the trend of floral change.

A comparison of the modern flowering plants with those of the Lower Eocene, which must be about 60 million years old, ought to provide significant results. From the London Clay, Reid and Chandler (53) have recognized about 314 species of fairly well preserved fruits and seeds, 59 could not yet be referred to a family and 234 were given specific names. The genera distinguished numbered about 100, belonging to 43 families, and 61 genera could be closely compared with 53 modern genera. If the floral structure of these modern genera is analyzed, we find the forms with perigynous flowers or with stamens inserted on a disc vastly outnumber the hypogynous forms with a convex torus; in fact, the genus *Magnolia,* represented by 10 species, is the only one whose flowers are

of the supposed primitive type. The best represented families are the Lauraceae, Icacinaceae, Euphorbiaceae and Anonaceae, while 7 sympetalous families are represented. Furthermore, the Nymphaeaceae is represented only by a form allied to *Barclaya,* the one member of the existing family which has perigynous or epipetalous stamens.

Following back the record of flowering plants to the Upper Cretaceous rocks, we find large floras in different parts of the Northern Hemisphere in which conifers played an important part. Unfortunately we can no longer place such implicit reliance on our identifications because they are based mainly on leaf impressions, but the general support recently given to the identifications of Tertiary leaves as indicated above, and the occasional discovery of recognizable fruits and seeds, suggests that the published lists of Cretaceous plants may give us a fairly accurate picture of the flora. The most important recent works have come from Berry (12–15) and Hollick (33) in North America. Seward has reinvestigated the flora from Greenland (61) while work has been done on a Central European flora of Cenomanian age from Czechoslovakia (74). From these papers it is clear that palms and certain other monocotyledons grew with a considerable variety of dicotyledons. The larger American collections suggest the presence of some 30 families in 20 or more orders, and, while a considerable number of forms appear to belong to extinct genera, most of them are thought to be near enough to modern genera to allow their attribution to families. If this has been done correctly, sympetalous forms are represented by about 10 per cent of the dicotyledons in each flora. There still seems to be no marked preponderance of genera with any single type of flower but the Polycarpicae of Wettstein are especially well represented. The Lauraceae are noticeably abundant while Magnoliaceae and Menispermaceae are also prominent. Sapindales, Rhamnales and Umbellales are thought to be well represented. In each flora many leaves are attributed to *Ficus,* and at several localities the remains of fruits have been discovered, while leaves with fruits referable to *Artocarpus* have also been recorded. Two families now especially characteristic of the Southern Hemisphere are frequent in Europe and North America, the Myrtaceae, on the evidence of leaves and of fruits identified as *Eucalyptus* (73), and the Proteaceae on the evidence of leaves, and the remains

of inflorescences (75). An independent line of evidence comes from the discovery of petrified wood. Specimens from North America, Europe, Egypt and Japan have been compared with the groups Caesalpinioideae, *Euonymus, Fagus, Juglans, Laurus,* Piperaceae, *Nothofagus, Phyllanthus, Populus, Rhus,* Celastraceae, *Sabia,* Saururaceae, Sterculiaceae (19). While the value of these comparisons is doubtful they should at least indicate that the flora was of a varied character. Our direct knowledge of flowers from this period is small but cannot be neglected. Stopes and Fuji (64) described a petrified form from Japan which had a trilocular ovary not more than 3 mm. high and slightly inferior, a perianth or disc being fused to the lower part of the ovary. Flowers or fruits of twelve different forms have been recently described from the Cenomanian of Czechoslovakia (74). Some of these have been compared with fruits or flowers of *Rhizophora* and *Celastrus.* The types *Rutaecarpus* and *Ceratocarpus* had syncarpous ovaries. *Triplicarpus* is figured as a whorl of fruits suggesting affinities with the Anonaceae, while fossils compared with flowers of *Myrica* and *Sparganium* have also been figured.

The records of the Lower Cretaceous rocks are interesting for the lower strata, such as the Patuxent series of America and the Wealden of Europe, contain only typical Mesozoic floras. There are no indubitable dicotyledonous leaves though three forms, called *Proteaephyllum, Ficophyllum* and *Rogersia,* have reticulate venation, possibly suggesting angiosperm affinities (11). The uppermost strata, however, contain an appreciable percentage of forms referable to the flowering plants and these again show considerable variety and seem to resemble a wide range of families. Some years ago, Saporta (56) identified about twenty genera of dicotyledons from the Albian rocks of Portugal on leaf impressions comparable with *Cissus, Magnolia, Nelumbium, Eucalyptus, Sassafras, Laurus, Myrica, Salix* and other forms. From the Patapsco beds of Maryland, Berry (11) described three fossils regarded as monocotyledonous together with a variety of dicotyledonous leaves. The names given to these types indicate their general aspect and these are *Populus, Populophyllum, Nelumbites, Menispermites, Sapindopsis, Celastrophyllum, Cissites, Sassafras, Araliaephyllum.* In addition, there were five genera of unknown affinities, possibly dicotyledonous. It is impossible to say whether the implied affini-

ties of these leaves can be substantiated, but there seems to be no reason why they should not be generally correct. In any event, we see that from the time when the angiosperms first became dominant in the floras of the marshes or estuaries they were represented by a wide range of types, clearly belonging to several divergent families. This conclusion is still more firmly established by our knowledge of Lower Cretaceous petrified woods. From beds of Senonian age come types named *Carpinoxylon, Cornoxylon, Fegonium, Juglandinum, Laurinum, Platanium, Salicinum* and *Taenioxylon,* from the Cenomanian were derived *Hamamelidoxylon* and *Salicinum,* from the Albian a *Laurinum* and from the Aptian, *Aptiana, Cantia, Woburnia* (*Dipterocarpoxylon*), *Hythia* and *Sabulia.* The Aptian woods from the Lower Greensand of England are among the earliest known dicotyledons of Europe and it is interesting to note that they were described by Stopes (62) as highly specialized types showing "little evidence of any primitive features." On the other hand, a number of these Lower Cretaceous forms show scalariform perforations, and several may be described as of a generalized type which appears today in such families as the Myricaceae, Theaceae, Myrtaceae, Tiliaceae, Ericaceae, Symplocaceae, Rubiaceae and Caprifoliaceae (2). It should be noticed that there is independent evidence from leaves or fruits suggesting the presence of several of these families in the Cretaceous or Lower Tertiary periods. In far eastern Siberia a few angiosperm-like leaves, named *Aralia, Proteaephyllum* and *Pandanophyllum,* appear in Aptian beds associated with Wealden species (45).

The study of Cretaceous floras seems, then, to indicate the sudden rise to dominance of angiosperms during this period, and has given rise to the view that about this time the flowering plants evolved with extreme rapidity from some unknown but widely different ancestors; but the facts do not justify this conclusion. All we can say is that during this period the angiosperms replaced many of the older gymnosperms in the floras of the estuarine and marsh lands. They must have long existed on dry ground and their spread may have been accelerated by the appearance of birds, or by some other biological factor. There is also the possibility that the distinctive dicotyledonous type of leaf was steadily evolving at this time from some other types which may have been found in older rocks but have not suggested angiospermous affinities. The far

greater antiquity of the group is proved by a few rare finds from Jurassic and Triassic rocks. Seward (58) described a leaf from the Stonesfield Slate (Jurassic) of England. A leaf (55) from the Solenhofen beds in Germany has an outline more like that of a compound dicotyledonous form than anything else. A piece of silicified wood (42) from the Brown Jura of Germany seems to have vessels with the ring-pore arrangement and may be a dicotyledonous type. While a silicified wood from India, probably but not certainly Jurassic, has no vessels but is comparable with the homoxylous dicotyledons in structure (54). This last example might, of course, belong to some gymnosperm at present unknown or to one of the Bennettitales. Its affinities and signification have given rise to some discussion (24, 79). Beyond the Jurassic the rocks of the Trias have furnished two interesting specimens. *Furcula* is a narrow leaf with a forked lamina, a reticulate venation and stomata of the angiospermous type, which was found by Professor Harris in Greenland (27). From rocks in South Africa, thought to be of a slightly older date, comes a structure not yet described in print and which, though not well preserved, can be compared only with an inflorescence bearing flowers. These have a whorled perianth of five (or possibly six) segments and a short or disc-like receptacle. Neither stamens nor carpels can be made out, but even in the absence of conclusive evidence the mere existence of structures of this type as early as the Middle Trias is most interesting and raises fresh doubts about the validity of the classical theory of the flower.

Professor Wieland has suggested dicotyledonous affinities for the winged seed or fruit called *Fraxinopsis* (78) found in the Rhaetic of Argentina, and later in Japan and Australia. A mere external similarity to *Fraxinus* does not, however, enable this suggestion to be received with confidence, especially in the light of recent discoveries of new plant groups in the Mesozoic. The Bennettitales and Caytoniales were of some importance in the Mesozoic floras but when we are working backwards it is very difficult to connect them directly with flowering plants, though they may well be distantly related through common ancestors. The Caytoniales show little or no approach towards flower formation though they were angiospermous in the Jurassic, while the Bennettitales, which had flower-like aggregations of their fertile organs, differ in most

of their structural details from the flowering plants and were undoubted gymnosperms.

The attempt to trace the history of the flowering plants backwards makes it clear that their ancestry must go far back to the early Mesozoic or Palaeozoic period. The historical sequence of forms shows that evolutionary change has been very slow, at least in many groups. None of the evolutionary schemes hitherto proposed for the dicotyledons seems to agree with the historical evidence which, moreover, gives no support to the current interpretation of floral morphology. On the other hand, we find that plants with small carpels, with whorled stamens and with the receptacle forming a disc-like or perigynous structure predominated among the earliest known floras. If any backward convergence of type is discernible, it is toward forms of this construction.

FOSSIL PLANTS SUGGEST A NEW VIEW OF FLORAL EVOLUTION

The difficulty of tracing backwards the history of a group is comparable to that experienced in finding the source of a river by travelling up from its mouth. Innumerable routes must be fruitlessly followed before the main stream can be distinguished from the lateral branches. More satisfactory results may be achieved by traversing the watershed and noting the general way in which the streams are flowing from the higher levels. So also our present quest may be advanced by studying first the oldest known floras and then trying to trace the general trends of evolutionary change accomplished with the passage of time. It is possible that in this way the floral and vegetative peculiarities of the angiosperms may appear in a new light, and we may be led to make comparative studies of features hitherto neglected.

Recent research (47, 46, 38, 43, 44) shows that the earliest known land plants in several distinct groups, which flourished some 400 million years ago, bore their sporangia at the ends of branches, and there seems to be no direct association of sporangia with foliar structures. Few, if any, plants could be described as possessing foliar sporophylls. Further, some of the oldest forms of Silurian age (47, 18A) possess terminal aggregations of free or united sporangia, which suggest that the sorus may be as old as the strobilus. It is likely that *Aneurophyton* (43), which may well be one

of the oldest known seed plants, bore whorls of pollen sacs at the tips of some of its branches.

As time progressed, the flattened photosynthetic branches of some seed plants became specialized foliar structures with an expanded compound lamina, but the terminal and whorled grouping of their pollen sacs persisted. The fertile branches may have formed part of a large foliar frond as in *Archaeopteris* (59), which was probably, but not certainly, a pteridosperm; however, in other types the fertile and vegetative branches may have remained distinct. The Lower Carboniferous rocks provide us with richer floras and here the forms called *Telangium* and *Scheutzia* by Kidston (37) as well as their contemporary ferns bore whorls or tufts of sporangia at the ends of naked branches. The seeds of these early forms also sprang from the ends of branches, either singly or in groups (1; 37, *464*; 9; 10). The fronds and petrified stems from the Lower Carboniferous show that a large number of pteridosperms were then living whose reproductive structures are as yet quite unknown. From the Upper Carboniferous rocks our knowledge is more extensive. In the coal balls we find petrified specimens of the true *Telangium* type (8) where terminal whorls of pollen-bearing sporangia are fused together at the base (Fig. 1, A), while at least two or three other types (Fig. 1, B–D) with the same general construction are known but have not yet received full study (57, *79*).

The well known *Crossotheca* (Fig. 1, E) known from moulds and carbonized specimens should probably be described as having whorls of bilocular sporangia borne on a disc-like receptacle terminating a fertile branch (35, 36, 18) though the sporangia have, under the influence of old ideas, been usually spoken of as borne on the margins of a sporophyll (57, *77*). The American *Codonotheca* belonged to this type of structure. But by this time the reproductive structures were beginning to diverge from the older and central type. Halle (26) has displayed a series, the Whittleseyinae, in which the sporangia of terminal whorls were concrescent and formed a cylindrical synangium varying in form in the different genera (Fig. 1, G, H). Then Halle also studied *Potoniea* (Fig. 1, F), the probable male flower of the Neuropterids, and here found a large number of free elongated sporangia borne on a cup-like receptacle at the end of a branch. *Potoniea* shows considerable

FIG. 1. Typical pollen-bearing organs from the Upper Carboniferous period. A–D, Petrified forms from English coal balls. A, *Telangium Scotti* Benson. B–D, Undescribed forms not yet fully studied. E, *Crossotheca Hoeninghausi* after Kidston. F, *Potoniea adiantiformis* after Halle and Kidston. G, *Whittleseya elegans* after Halle. H, *Aulacotheca elongata* after Halle. I, *Zeilleria avoldensis* after Kidston. J, *Dactylotheca plumosa* after Zeiller. All figures diagrammatic and not drawn to scale.

morphological similarity to the male flowers of *Populus, Mollinedia* and other modern forms. In some pteridosperm groups the fertile branches appear to have taken part in frond formation as in the ferns. *Zeilleria* (Fig. 1, I) was one of these and had spherical synangia terminating the main marginal veins of a dissected frond (26), while *Dactylotheca* (Fig. 1, J) had superficial sori of elongated free sporangia (*37, 382*). Pteridospermous plants are now known to have survived into the Mesozoic (69, 27). Their pollen was produced in elongated unilocular or bilocular sporangia produced in groups or tufts on special branches but showing varied modifications. An expanded receptacle of the *Crossotheca* type is found in *Pteruchus* (69) [Fig. 2, A], but the sporangia may be more or less spread along the fertile limb as in *Lepidopteris* (27)

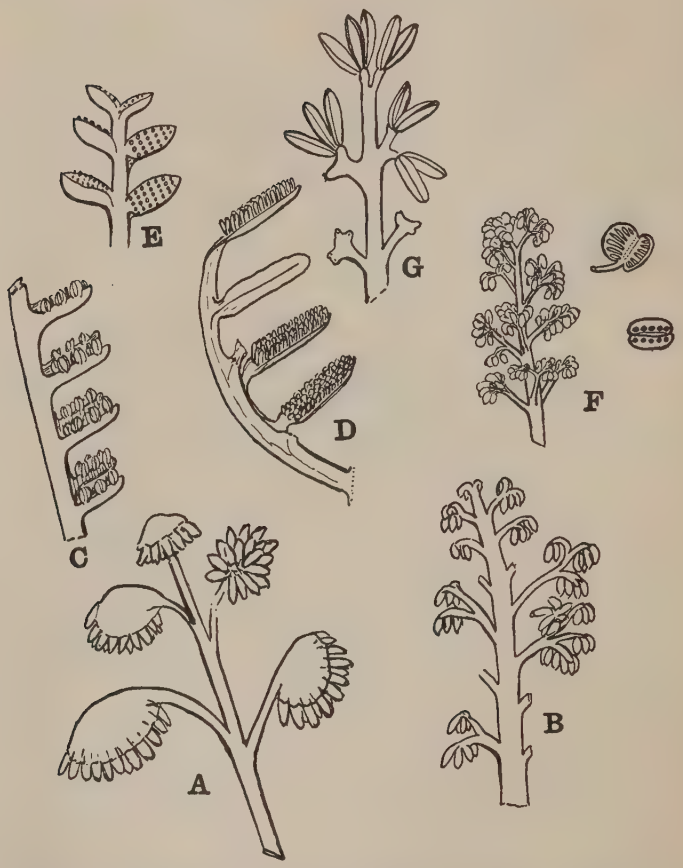

FIG. 2. Pollen-bearing organs from the Mesozoic period. A, *Pteruchus africanus*. B, *Antholithus* (*Lepidopteris*) *Zeilleri*. C, *Lunzia austriaca*. D, *Westersheimia pramelreuthensis*. E, *Bennettistemon amblum*. F, *Hydropteridangium marsilioides*. G, *Antholithus* (*Caytonia*) *Arberi*. B, E, F, after Harris; others original. The figures are not drawn to scale.

[Fig. 2, B] and *Lunzia* (40) [Fig. 2, C]. In this way a distinct form of sporophyll is reached. Several little-known Triassic forms, such as *Westersheimia* (40) [Fig. 2, D], show a fuller development of this tendency which leads on through *Bennettiste-*

mon (28) [Fig. 2, E] to the Bennettitales. The morphology of the Jurassic and Cretaceous male flowers (76, 77, 48, 49, 67) of this group presents a difficult problem. The well known and characteristic synangia must be considered in relation to *Hydropteridangium* (28, *122*) [Fig. 2, F] but the free or concrescent structures which bore them invariably formed a whorl and no tendency towards a spiral arrangement has been yet found. The angiosperm-like anthers of the Caytoniales (68) were produced in groups or singly on branching pinnate structures [Fig. 2, G], which, like those of *Pteruchus* and *Lepidopteris,* may be described as sporophylls, but must have been derived from flattened branch systems. Such structures may have a bearing on the branched stamens found in such plants as *Ricinus, Hypericum* and *Calothamnus,* but raise grave doubts as to the propriety of describing all angiospermous stamens as microsporophylls.

The earliest known seeds terminated branchlets [Fig. 3, A–C] and subsequent changes in the position of the seeds are often parallel to changes exhibited by the pollen sac groups. Recent research tends to favor the view of the origin of seeds from a sorus in which a central sporangium only remains fertile (8) while the peripheral sporangia were sterilized to give the integument (and perhaps also the cupule). Many forms of Carboniferous seeds are known [Fig. 3, A–G] but there is very little evidence as to their position on the fertile branches. Grand Eury (23), after many years of field work, expressed the view that most pteridospermous seeds were borne on special branch systems and not on foliage fronds.

It may be significant that the few examples of seeds borne on photosynthetic fronds have been found mainly in the youngest of the Palaeozoic strata (25, 37, *400*). The evidence suggests that fronds bearing marginal or superficial seeds were more usual in the Permian period, but the later Triassic forms show a complete separation of fertile and vegetative structures. There can be no doubt that the seeds of the Corystospermaceae [Fig. 3, J–L] and Peltaspermaceae (69) [Fig. 3, M] were produced on separate branch systems which, having bracts and bracteoles, have been described as single inflorescences. There is no certain evidence of the production of seeds on the margins of a flattened foliar lamina from any Mesozoic rocks. The megasporophyll of *Cycas* has often

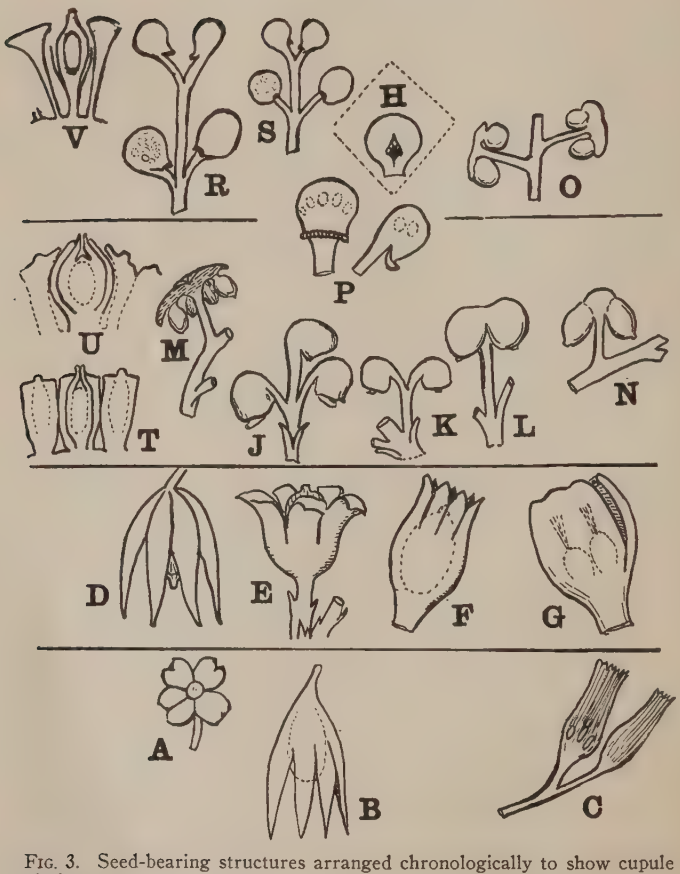

FIG. 3. Seed-bearing structures arranged chronologically to show cupule evolution.

UPPER DEVONIAN AND LOWER CARBONIFEROUS. A, *"Telangium" bifidum* after Kidston. B, ? *Archaeopteris* after Arnold. C, *Calathiops Bernhardti* after Benson.

UPPER CARBONIFEROUS. D, *Sphenopteris striata* after Bertrand. E, *Lagenostoma Lomaxi.* F, *Lagenostoma Sinclairi* after Arber. G, *Gnetopsis elliptica.*

TRIASSIC. J, *Pilophorosperma granulatum.* K, *P. gracile.* L, *P. geminatum.* N, *Nilssonia incisoserrata.* M, *Lepidopteris.* P, *Caytonia Thomasi* after Harris. T, *Williamsonia Wettsteini.* U, *Vardekloeftia* after Harris.

JURASSIC. O, *Beania gracilis,* probably an early cycadean form. R, *Gristhorpia Nathorsti,* with closed ovary and large stigma. S, *Caytonia Sewardi,* closed ovary and small stigma. V, *Bennettites,* and *Williamsonia* with much reduced cupule. H, Hypothetical early angiospermic carpel formed from two concrescent cupules with lateral stigma.

All figures diagrammatic and not drawn to scale.

been used in discussions on carpel morphology, and the fossil *Cycadospadix* (*Palaeocycas*) has been compared with this structure, but Florin (22) has shown that this structure differs considerably from the modern *Cycas* and it may be here remarked that no fossil specimen has ever been found with an attached seed. On the other hand, the Triassic and Jurassic structures named *Beania* (Fig. 3, N, O) suggest that the flattened megasporophyll is not necessarily a primitive type of structure in the cycads.

The Jurassic Caytoniales seem to show how angiospermy actually came about in a group of plants, descended from pteridosperms of the *Pilophorosperma* type, but quite distinct from and probably contemporary with the earlier angiosperms. A Triassic member of this group (29) was a gymnosperm (Fig. 3, P) for the pollen grains reached the micropyles of the seeds. The later Jurassic members (Fig. 3, R, S) were angiosperms with the pollen grains adhering to a stigma and never reaching the ovules directly (68). The ovary wall must here be related to the cupule of the Palaeozoic seeds, and a number of seeds was produced within it. Thomas has suggested that the carpel wall of the angiosperms may represent a pair of concrescent cupules (70, 72) and that the possible origin of the stigma should be considered in the light of these ancient forms (72). In earlier papers of this author it was assumed that the current morphological interpretations of stamens and carpels were correct, but subsequent consideration of the foundations of the classical theory (71) have caused important modifications of his earlier views; however, the idea that the angiosperms, Caytoniales and Bennettitales arose from a common ancient plexus is still maintained.

The present position is that the fossil record seems to provide material for a completely new view of the morphology of the closed ovary and stigma. This view needs to be tested without preconceptions by comparative studies of modern forms.

The Mesozoic Bennettitales diverge widely from the angiosperms in the construction of their ovuliferous parts, but must be considered in relation to floral morphology (1A). In this group we find reproductive structures closely aggregated at the top of a longer or shorter axis, showing no indication of strobilar structures in their early stages (76, *178*, 63, *431*). Some genera show unisexual and others bisexual aggregates, often with a number of

lanceolate structures like a perianth. The upper part of the fertile axis bears a very large number of appendages packed together to form a spherical or conical mass, and differentiated into stalked ovules and sterile interseminal scales. Some of the older forms (Fig. 3, T) from the Trias (41) give strong indication that the interseminal scales represent aborted ovules and certain later examples (28, *112,* 63) (Fig. 3, U) support this interpretation. The so-called female strobilus would then represent a terminal tuft of ovules in which little or no trace of regular spiral arrangement is discernible. But the structure of these ovules shows strong affinities with the pteridosperm seeds, and it is known that the earlier forms possessed cupular envelopes which became much reduced in the later forms. We thus get no approach to the expanded foliar carpel of the older authors, but we have to deal simply with a mass of gymnosperm ovules at the apex of a shoot. If this is the true interpretation of a flower-like structure which lasted for a very long time, it seems possible to apply it to certain angiospermous flowers which show carpels crowded on the floral axis. It is at least possible to consider that the earlier ancestors of some flowering plants may have had a terminal aggregate of short stalks each bearing *a pair* of cupules enclosing two or more ovules which by the concrescence of the cupules gave rise to closed ovaries. Furthermore, it is possible that the process which resulted in the production of bisexual bennettitalean flowers from the unisexual groups of reproductive structures found in the older pteridosperms may well have given rise also to the bisexual flowers of the angiosperms. The nature of this process awaits further discoveries in the field of recent taxonomoy, cytology and genetics, as well as the investigation of the much neglected physiology of reproduction.

In concluding our historical survey of the reproductive structures of megaphyllous seed plants, we cannot avoid certain conclusions:

(a) That while no support can be found for the classical views of floral morphology, the consideration of all the known forms in their chronological sequence makes inevitable a new concept of floral structure.

(b) The evidence derived as we progress from a period some 400 million years ago to the present day provides material for a consistent and physiologically possible theory of floral evolution which accords with what we know of the Cretaceous and early Tertiary angiosperms and seems applicable to the flowers of to-day.

(c) The flower seems to have commenced as a sorus or tuft of sporangia terminating a branch before any specialized foliar structures had been evolved.

(d) Concrescence of the elongated pollen-bearing structures was an early feature while the apex of the stem often appears expanded to a disc-like receptacle. Several Coal Measure pteridosperms had male flowers morphologically similar to those of *Populus* but their anthers were uni- or biloculate.

(e) The concept of the stamen as a complete microsprophyll seems untrustworthy.

(f) Seeds arose singly on branch endings, but soon show aggregations. Single seeds or groups became enclosed in an envelope, the cupule, which covered them more and more completely as time went on and often became recurved.

(g) Pairs of cupulate seeds sometimes grew on short branches of an "inflorescence," and in the early Mesozoic may show a partial fusion of the two cupules.

(h) In one group the cupules became closed and the pollen grains were deposited on their papillate margins instead of reaching the micropyles directly, so effecting a change from gymnospermy to angiospermy.

(i) The carpels of flowering plants may then possibly represent two fused cupules rather than a single foliar structure. The more primitive carpels would thus be small almost spherical structures with an extended stigma arising on their ventral side (Fig. 3, H).

(j) In different groups and at different periods cupulate seeds or seeds enclosed in an ovary appeared in the center of the receptacle or disc bearing pollen sacs.

From the fossil evidence a picture of floral evolution can be constructed in which almost every stage is represented by an actual plant structure dating from about the period when we should expect it to occur.

It remains to be seen whether these concepts are fully applicable to modern genera, but they have obvious advantages over the classical views which involved the postulation of an ancestral gymnosperm differing from any known plant, and a course of evolution for which there is no evidence. Botanists may be reluctant to give up views which have been passed on by so many generations of investigators, but if so they must provide the classical theory with objective foundations. For instance, they must provide good reasons for believing that modern flowers have arisen by the condensation of an elongated floral axis and not from a terminal sorus as has been suggested above. However, we have several independent sources which ought to provide means of testing the theories

of the comparative morphologist as to the nature of primitive flowering plants and thus a wide field is open for future investigation.

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APPENDIX

Geological Time Scale

Period		*Approximate age in millions of years*
Upper Tertiary	Pleistocene Pliocene	30
Lower Tertiary	Miocene Oligocene Eocene	60
Upper Cretaceous	Senonian Turonian Cenomanian	90
Lower Cretaceous	Albian—(Patapsco) Aptian Neocomian (Wealden, Patuxent)	120
Jurassic		150
Triassic		180
Upper Permian		210
Lower Permian or Permo-Carboniferous		240
Upper Carboniferous		270
Lower Carboniferous		300
Upper Devonian		360
Middle Devonian		390
Lower Devonian		420
Silurian		450
Lower Cambrian with first-known fossils		600

Explanatory Notes

angiospermy: the condition of ovules being borne within a closed structure, the ovary. Applies to all modern seed plants except the Ginkgoales, Cycadales, Coniferales and Gnetales which are gymnosperms.

coal-balls: calcareous nodules containing petrified patches of vegetable debris from the marshes and swamps of the closing stages of the Carboniferous period (Seward).

concrescence: a growing together.

cupule: a free sheathing structure from the peduncle investing one or more seeds (Oliver & Salisbury).

epigynous: refers to flowers whose stamens stand on the pistil, apparently above the ovary.

gymnospermy: the condition of ovules being borne, not within a closed ovary, but uncovered. See angiospermy.

homoxylous: plants with wood of uniform structure and which (like the pines) contains no vessels.

hypogynous: refers to flowers having stamens inserted below the ovary.

megaphyllous } in evolutionary studies plants have been divided into the
microphyllous } small-leaved (micro-) such as mosses and lycopods, and the large-leaved (mega-).

perigynous: refers to flowers having stamens on a rim-like expansion of the receptacle or on the perianth. Intermediate betwen hypogynous and epigynous.
pteridosperm: prehistoric fossil plants, fern-like in foliage but with gymnosperm-like reproductive organs.
receptacle: the portion of a flower axis which bears the floral organs.
ring-porous wood: wood in which the pores (vessels) of one part of a growth ring are in distinct contrast in size or number (or both) to those of the other part.
scalariform vessel: a vessel having elongated perforations or thickenings showing a ladder-like arrangement, like those of woody elements of ferns.
sorus: a cluster of sporangia in ferns.
sporangium: a sac producing spores within it.
sympetalous: with united petals. Generally regarded as an advanced type.
synangium: an aggregate of united spore-sacs formed either by the union of several distinct sporangia or by the development of separate chambers in a single sporangium.
strobilus: a cone-like structure made up of overlapping scales (sporophylls) bearing reproductive organs.

PLANT TISSUE CULTURES

PHILIP R. WHITE

The term "tissue culture" is too well known to the scientific public through the work of Harrison (56–59), Burrows (10), Carrel (11–15), Fischer (31–36), Erdmann (25, 26), Lewis and Lewis (78–80), and others to need definition. In general, it has been used in such work to designate preparations in which somatic cells of a single type or a restricted number of types (fibroblasts, osteoblasts, epithelial cells, etc.), isolated from an animal body, have been kept growing for more or less extended periods of time, *in vitro*.

The concept of potentially unlimited growth as a *sine qua non* of a tissue culture is, in general, adhered to by Carrel and his school, but rejected by Lewis and others. Likewise, the concept that a tissue culture should be of a single type of cell and should undergo only limited differentiation is generally adopted by Carrel (13, 15), but must be rejected in the application of the term to the "organule" cultures of Fischer (31, 32, 34), Fischer and Parker (37), Fell (29, 30), *et al.* In view of this divergence of opinion, even in the animal field, as to just what constitutes a "tissue culture," and in view of the comparative newness of the term in the working vocabulary of most botanists, it seems desirable to begin by setting forth a definition for use in the following pages. A plant tissue culture will hereafter be considered to be *any preparation of one or more isolated, somatic plant cells which grows and functions normally, in vitro, without giving rise to an entire plant.* Under such a definition, spore cultures, cuttings, and cultures of whole embryos will be excluded, but a limited amount of differentiation will not be considered as automatically excluding a particular culture from this category. It is realized that such a definition may raise objections in some minds, and suggestions for its improvement will be welcomed. The author is inclined to concur in Carrel's concept that unlimited growth is a necessary criterion to be included in a proper definition, but since such an interpretation would restrict the literature of the field to only a very few papers, it will not be insisted upon at present. Nevertheless, it should be kept in mind that *unlimited survival* and growth has been the *aim*

of most of the work in both the animal and plant fields, and that one of Carrel's chief contributions to the field of animal tissue cultures was the development of a nutrient which would permit this aim to be attained (14).

Until recently, active interest in the subject of plant tissue cultures has been restricted almost exclusively to Germany, and publication has been mostly in non-botanical journals. For early discussions of the problem the reader is referred to the résumés of Börger (7), Küster (70, 71), Lamprecht (76), Schneider (107) and White (123), and the historical introductions to the memoirs of Gautheret (44) and Scheitterer (104).

Certain phases of botanical research not falling strictly in the category of tissue cultures have, nevertheless, been important in laying the foundations of technique and knowledge of tissue behavior necessary for success in the more specialized field, and should be briefly mentioned. Studies in tissue repair in injured plant organs (Vöchting, 119, 120; Hanstein, 55; Jaeger, 62; Küster, 68; Schilling, 105; La Rue, 72; Reiche, 98; Lopriore, 81), of callus formation and abnormal proliferation (La Rue, 73; Němec, 89; Stingl, 111; Haberlandt, 52, 53; Okado, 90; Dale, 20; Küster, 69; von Schrenk, 108; Winkler, 132), and of wound healing in general (Haberlandt, 52, 53; Brieger, 8; Lamprecht, 75; Miehe, 86; Olufsen, 91), have added considerable information to our store of knowledge of the conditions necessary for tissue proliferation. The cultivation of isolated embryos (Andronescu, 1; Arnaudov, 2; Tukey, 116, 117; Dietrich, 23; Essenbeck u. Suessenguth, 27; Buckner and Kastle, 9; Hannig, 54; Stingl, 110; White, 124) and the aseptic cultivation of whole plants (Combes, 18; McMillan, 85; Klein u. Kisser, 64; Bobko, 5; Hatch, 60; Weissflog, 122; Gerretsen, 45) have aided in determining the necessary environmental conditions and the experimental techniques adapted to such work. The extensive literature on the rooting of cuttings has likewise contributed much to our background.

While it is thus possible to mention a great deal of literature collateral to the subject, that referring strictly to plant tissue cultures, as such, as defined here, is quite meagre. Any treatment of the subject must begin with the work of Haberlandt (46–53), reported during the first third of the present century. Haberlandt clearly formulated the problem in 1902 when he said (46):* "So

* Reviewer's translation.

far as I know, there has been, up to the present, no well planned attempt to cultivate the isolated vegetative cells of higher plants in suitable nutrients. Yet the results of such cultures should throw many interesting side-lights on the peculiarities and capacities of the cell as an 'elementary organism'; they should bring into evidence the reciprocal relationships and many-sided influences to which the individual cells of a multicellular organism are subjected." This is, so far as the present writer is aware, the first formulation of the tissue culture idea, antedating by several years the first work in the field of animal tissue cultures (Harrison, 1907 (56), Burrows, 1910 (10), Carrel, 1911 (11)). Haberlandt attempted at the very outset to cultivate single cells, using palisade and medullary parenchyma, trichomes of various types (glandular hairs, stinging hairs, stamen hairs), epidermal cells, etc. (46). Although a perusal of this and subsequent papers makes it evident that he and his pupils grasped very well the nutritional problems involved, they seem not to have appreciated the important growth-restricting effects of differentiation, especially as regards the deposition of cell walls. The experimental results obtained were not encouraging. These investigations were continued in Haberlandt's laboratory over a long period of time, by Bobilioff-Preisser (4), Lamprecht (75), Thielmann (112–114), Thielmann and Bérziň (115), and others, and independently in other laboratories the same sort of approach has been used by Pfeiffer (95, 96), La Rue (72–74), Scheitterer (104), Úlehla (118), Schmucker (106), Kemmer (63), Börger (6), Czech (19), Winkler (132), and Kunkel (67). These authors never obtained definitely demonstrable, continued proliferation, and in 1927 Küster reviewed this work with the statement that (71)* "never to this day have isolated (plant) cells been brought to reproduce their tissues; all attempts have given the same negative results."

Molliard (88), Dauphiné (21), Rechinger (97), Scheitterer (104), and Smith (109) have attempted to grow small fragments of various tissues, especially pieces of embryos, with little success. The only work of this sort which appears to have been at all successful is that of Behre (3) with tissues of *Drosera* and certain unpublished work (1936) of La Rue (74), which cannot as yet be properly evaluated.

*Reviewer's translation.

From a careful study of these papers, the present reviewer feels justified in saying that up to 1936 only two groups of work in the field have given real promise of success. These two have taken their initial inspiration from the laboratories of Haberlandt (Berlin) and Molliard (Paris), and have dealt, respectively, with apical and with lateral meristems. Because it can be treated more briefly, the second mentioned work will be discussed first.

In 1935 Gautheret published an extensive memoir (44) entitled* "Researches on the cultivation of plant tissues," in which some 50 pages are devoted to a discussion of experiments on the cultivation of cambium removed from large trees of various species. The same work was summarized briefly in an earlier paper (43). Fragments of cambium a centimeter or more on a side were excised under aseptic conditions and placed on cotton or gelatin saturated with a nutrient solution. A slow proliferation was obtained giving rise to loose pseudo-parenchymatous outgrowths resembling the thalli of *Pleurococcus*. Growth continued over several months. These proliferations were similar to those described in regenerating tissues by Hanstein (55) in 1882. Histological preparations showed extensive modification of structure, not only of the surface layers but of more deep-seated tissues as well, with formation of an undifferentiated tissue resembling, at the exposed surface, that occurring in lenticels (Hanstein, 55) and, in the deeper tissues, that formed from proliferating pericycle and medullary rays or in the healing of internal wounds, as observed by Jaeger (62). Mitotic figures occurred frequently. The conditions employed did not permit indefinitely prolonged growth, so that this work does not fulfill that criterion of a tissue culture, and it is rather uncertain whether the changes occurring can be interpreted as those normal to a healthy tissue released from the influence of surrounding tissues, or merely those characteristic of injured cambium and cortex, left *in situ*. Moreover, only a small part of each explant took part in the growth, the major portion remaining dormant or becoming necrotic, so that it is uncertain if these cultures actually drew any of their material for growth from the nutrient supplied. The work will have to be greatly extended before it can be properly evaluated. Nevertheless, the result is promising and is important as representing the *only* case in the entire literature of the subject

* Reviewer's translation.

which would fulfill Carrel's second requirement, of *undifferentiated* active growth. In the strict sense, this is thus the only work reported to date that can even tentatively be called a "tissue culture" of plant material, and even this, as pointed out above, is a somewhat doubtful case. Further work in this direction will be followed with great interest.

This study of Gautheret represents the only work with the second category of tissues mentioned above, lateral meristems (cambium), which gave promise of success. The other, and in the present writer's opinion more promising and much more extensively developed approach to the tissue culture problem, has been that with isolated terminal meristems—stem-tips and root-tips.

The culturing of stem-tips is mentioned briefly by Robbins (100) and is treated in more detail by White (127). Using stem growing points of *Stellaria media,* the latter obtained considerable growth and differentiation, with apparently normal photosynthesis. The complex of conditions employed was not satisfactory for unlimited growth, and the normal mode of differentiation shown by these cultures suggested the behavior of cuttings rather than of tissue cultures, so that the work need not be treated in detail.

The cultivation of root-tips has been much more widely attempted and has given more satisfactory results. Heidt (61) and Felber-Pisk (28) studied the behavior of isolated roots in nonnutrient media or in air, and obtained only a very limited growth. Mayer (84) employed a great many different nutrient combinations, but obtained unsatisfactory results, probably due to excessive handling of the cultures with concomitant trauma. Really promising results with root-tips, however, were obtained by six authors, Kotte (65, 66), Robbins (100, 101), Robbins and Maneval (102, 103), Malyschev (82, 83), Gautheret (39–42), and White (124–126, 128–131). This work will require more extensive treatment.

In 1922 three papers on the cultivation of excised root-tips in nutrient media, representing independent work, appeared almost simultaneously: two by Kotte (65, 66) working under Haberlandt in Berlin, the other by Robbins (100) in the United States. Kotte grew apical and sub-apical fragments of *Pisum* and *Zea* roots on various media. He studied the nutrition of such root fragments, examining their requirements as regards sugars, organic nitrogen sources (amino-acids, peptones, tissue extracts), and inorganic

salts. He also studied their behavior toward light and gravity, the effect of size of initial fragment, of position of fragment in the root, of age of tissue, the phenomena of growth polarity, etc. He obtained considerable but not indefinite growth, some differentiation, but no suggestion of the formation of stem primordia. His best results were obtained with *Zea* roots in tubes of agar containing a Knop solution plus 1% dextrose with the addition of any one of four accessory materials which were, in descending order of satisfactoriness: 1) Liebig's meat extract; 2) α-alanine; 3) a mixture of peptone, asparagine, alanine, and glycine; 4) a pepsin-diastase digest of pea seeds. Such cultures grew well and branched actively for some time. Sub-cultures were not attempted and Kotte has published no further work on this subject.

In the same year Robbins, working independently, published (100) similar results. He grew roots of *Pisum, Zea* and *Gossypium.* He found a liquid medium somewhat more satisfactory than a solid one and studied the effects of several different sugars, concluding that dextrose was satisfactory for these plants. Roots were carried through three transfers (six weeks) but eventually died. In a second paper (101) the effects of various organic nitrogen sources were studied. The amino-acids were consistently found to be either detrimental or without effect. The best results were obtained with an autolyzate of yeast. This was studied at several concentrations. The same author and Maneval (102) extended the study to include tissue extracts and different concentrations of the nutrient ingredients, and in 1924 studied (103) the effect of light in the presence or absence of yeast extract. They concluded that moderate illumination was desirable. Many different sorts of plants were studied. All species, with the exception, curiously enough, of *Lupinus albus,* gave fairly satisfactory results. Some cultures were carried through ten passages (150 days), but eventually showed markedly decreased growth rates. These four papers (100–103), together with Kotte's two (65, 66), established a fairly secure basis on which to build, giving distinct promise of success.

Chambers' experiments (16, 17) in growing squash root-tips in hanging drops, in which he reported migration of cells as in animal fibroblast cultures, have not been confirmed, and were probably erroneously interpreted (see 123, 124).

In 1932 Malyschev, using a technique similar to that of Kotte, succeeded in carrying root cultures through 13 passages (83). He does not give details of growth rates, but made certain important observations on the differing carbohydrate requirements of different plants.

The major part of the history of this phase of the subject in the last ten years is, however, mostly the work of Gautheret and of White. In 1932 and 1933 Gautheret, who has already been mentioned in another connection, grew a number of sorts of roots and root fragments in various nutrients (39–42). An extensive account of this and other work was published in his memoir of 1935. All of his roots ceased to grow after a certain period varying from 10 to 100 days, and the author concluded that unlimited growth is not possible. Like many other authors, he has recourse, as an explanation for this failure of isolated tissues to grow indefinitely, to the theory of "hormones" provided by the parent plant, essential for growth and not formed in these tissues. Gautheret made a number of contributions to the technique of such cultures. In the opinion of the present reviewer, an important contribution was the demonstration that, although cysteïn-HCl is toxic at concentrations corresponding to those used by Robbins, Kotte, and other earlier workers in studying the effects of amino-acids, it is, nevertheless, markedly stimulating at lower concentrations, of 10^{-6} or less (44). Since neither unlimited growth nor undifferentiated growth was obtained, the results are, however, merely suggestive of certain potentially fertile methods of approach. Dauphiné (22) and Galligar (38) have made somewhat similar cultures.

In 1931 White began a series of studies in the general field of plant tissue cultures (123–130). In an introductory paper (123) the history of the subject was reviewed in detail. A brief paper (124) on the cultivation, in hanging drops, of three types of meristems: root-tips, embryos, and seed primordia, appeared in 1932. So far as the writer is aware, the only other paper in which attempts at cultivation of the last-named type of tissue are discussed is the unpublished dissertation of Moebius (Leipzig) (87). All three sorts of tissue gave distinct promise of success; only one, root-tips, has been followed up in detail, however. In 1933 appeared the paper on stem-tips (127) already mentioned. The cultivation of root-tips of *Triticum* was taken up in detail in 1932,

with a study of the effects of light, temperature, H-ion concentration, aeration, solution-volume, and presence or absence of dextrose or yeast or both (125). In 1933 the inorganic nutrient was carefully studied (126) with respect to each of its constituent ions, and a satisfactory formula developed. A very rapid and apparently normal growth was obtained for the short periods studied (two weeks only) in the best complex tested. The chief points of importance to be found in these papers are: the sharply-defined *maximum* temperature for growth (ca. 28° C.), the necessity for an *acid* solution (pH 5.0 to 5.5), in which respect these cultures differ from those of animal tissues which require a neutral or alkaline medium, the very low maximal as well as optimal concentration of phosphate (ca. 0.1 millimol as KH_2PO_4), the extreme sensitiveness of such cultures to absence or too low concentration of iron, the sharply-defined optima for calcium and potassium concentrations, and the relative indifference of the tissues studied to variation in anion concentrations. The nutrient developed in this work has been repeatedly compared with other well-known nutrients and found superior for such cultures (unpublished results). A brief note (128) on the effect of – SH compounds on root cultures, and one on the relative merits of liquid and solid media (129) likewise appeared in 1933.

Indefinitely-continued growth of such cultures was not obtained by Kotte, Robbins, Chambers, Malyschev, or Gautheret, and more or less elaborate theories, such as that of Miehe (86), have been evoked to explain this fact. The most widely accepted of these theories: that the plant must contain substances necessary for growth, which are irreplaceable and whose synthesis by isolated roots from the constituents of the culture medium is impossible, is perhaps best expressed by Gautheret (44, pp. 109–110, 1935). The arrest in development would, according to this theory, be the result of a disturbed equilibrium arising from the isolation and causing a complete change in the nutrition of the root. This has been the point of view of most of those who have worked in the field within recent years, and was justified by much of the data available until recently. In 1934, however, White (130), using tomato root-tips, a nutrient and culture method based on those developed in earlier papers (125, 126), with carbohydrate changed from dextrose to sucrose as required by tomato (unpublished re-

sults; compare Malyschev, 82), and employing certain slight modifications in method of preparing the nutrient, succeeded in carrying roots through more than 50 passages. A mean growth rate of ca. 5 mm. per culture per day (a quite normal value) was maintained, and a total *potential* tissue multiplication of the order of 10^{40} was obtained. The *actual* measured linear increment of a single isolated root-tip during one year of culture *in vitro* was 15,500 times the initial length, with 35,400 branches. This same root has now, at the present writing, been carried through about 160 passages (more than three years), has made a total measured increment exclusive of branches of nearly 2 miles, and is still growing entirely normally. The theory of irreplaceable essential substances expressed by Gautheret (44) and suggested as a possibility by Robbins (101) and others is evidently unnecessary, although "accessory substances" of an as yet unknown nature have been regularly supplied to these cultures in the form of a soluble extract of .01% of dried brewers' yeast (Harris). Since, however, Osborne and Wakeman (92) have shown that such an extract is free from demonstrable protein, these "accessory substances" cannot be very complex and should be capable of identification. In any case, they represent only a very minute fraction (less than 1/10,000) of the nutrient. An important feature of this work is that the medium used, unlike the media employed in animal tissue cultures, contains only this one unknown. All other constituents of the nutrient are known and can be varied at will.

Certain characteristics of these cultures are worthy of note. In routine work the initial explant has been regularly a piece 10–15 mm. long, including the apical meristem and some elongating tissue (130). This normally floats on the surface of the nutrient (125). Sometimes roots which at first sink to the bottom will subsequently come to the surface, but the growth rates and general appearance of the cultures are almost invariably better in those which have floated throughout the culture period (125). These must be considered to represent the normal type. This behavior of healthy tissues is probably due to occluded respiration gases. The excised root elongates as does a root in the soil, without any apparent abnormality except that, contrary to Kotte's findings (65, 66), it does not react geotropically. Five to 10 mm. of the tip of a growing culture may bend downward somewhat, but this curvature is

always removed in the region of elongation, so that a root grows horizontally throughout all but the terminal centimeter and continues straight as long as the restricting space allows, later curving around the inner surface of the flask. Whether failure to bend permanently geotropically is due to loss of reactivity, or merely to a balance between the downward geotropic tendency and the upward chemotactic reaction toward the oxygen gradient in the nutrient, or to the low specific gravity of the tissue which, in the absence of any firm point of attachment, prevents the geotropic reaction from being effective, cannot at present be said. Growth rates are somewhat variable but in general average an increase of about 5 mm. (one-half to one-third of the culture's original length) per day. A culture thus doubles in volume in the first 48 to 72 hours. This figure is comparable to that considered normal for animal tissue cultures (15). Individual growth rates as high as 40 mm. per day have occasionally been recorded, and one group of cultures under unusually satisfactory conditions averaged 13 mm. per culture per day for an entire week (unpublished data). Cultures usually begin to branch from the base on the third or fourth day, and at the end of a week will frequently have 30 or 40 branches, each one capable of being used as a sub-culture. The roots themselves are entirely normal in color; the root-cap is characteristic in its form and in its manner of sloughing off (124). The cells reported by Scheitterer (104) as simulating migrating cells (compare Chambers, 16, 17; Pfeiffer, 96; and White, 124) were apparently sloughed off root-cap and root epidermis cells. The main portion of the culture, contrary to the observations of Malyschev (82) (see Gautheret, 44, p. 99), maintains a constant diameter. If branch roots of small diameter are used as sub-cultures, they very quickly enlarge to this normal diameter as they grow. Because of this fact, linear increments are quite as satisfactory measures of tissue increase (125, 129, 130) as are dry weights. Cultured roots seldom exceed this norm, and when they do, are evidently unhealthy. Such excessively thick cultures do not grow satisfactorily. It is, therefore, clear that secondary thickening is not a characteristic feature of such isolated roots. Nevertheless, complete vascular strands containing all the primary elements are regularly formed (124. 130), although it would seem improbable that they perform either of their major normal func-

tions of conduction and support. No interfascicular cambium or pheloderm is laid down, but proliferation of the pericycle to form secondary growing points (branches) goes on normally. When a piece of root containing no apical growing point is cultured, callus is formed in considerable quantity at the distal end but not at the proximal (Kotte, 66; compare Vöchting, 120), and when branches are formed on such a piece their insertion on the root is characteristically oriented. The polarity of growth, as was pointed out long ago by Vöchting (120), is thus maintained. The root-cap cells contain normal quantities of starch and the older cortical cells at the base of cultivated roots, likewise, become loaded with starch. The development of these cultures, so far as it goes, appears to be quite normal both in quality and quantity. But, although *growth* is potentially unlimited (130), *differentiation* is not. These are typical rootlets, but in the absence of secondary thickening, cambium, pheloderm, etc., even in cultures maintained for months without transfer (130), they cannot be called "roots" in a complete sense. And since no stem-tips are formed, they are certainly not plants.

The question arises, are they "tissue cultures"? Strictly speaking, they are not. They are "organ cultures." Strictly speaking, "tissue cultures" of plants have never been obtained. Gautheret's cambium cultures (44), mentioned before, appear to have satisfied the criterion of undifferentiating growth. White's cultures have satisfied the criterion of unlimited growth. But both criteria have never been satisfied in any single instance reported to date. The consideration of these root cultures in a paper on tissue cultures must be justified on the basis of their aim, and the fact that the method of approach is certainly that which must be employed if tissue cultures are to be obtained. Furthermore, it is the writer's belief that there is a growing feeling among animal tissue culturists that "differentiation" is a matter of environmental conditions as much as it is of internal factors, and that the undifferentiating pure-line cultures of Carrel (13), Ebeling (24), Fischer (35, 36), Parker (94), and others have come to be considered the normal type only because of the aims in mind. Thus Parker, using a pure-line culture of blood monocytes, but replacing the usual solid medium by a liquid medium similar to that used by White and Robbins, caused these pure-line cultures to differentiate into

a capillarogenic tissue (93). The work of Carrel, Ebeling, and others has shown that heart fibroblasts, when grown in pure culture, maintain an undifferentiated behavior indefinitely. Cartilage cells derived from the embryonic eye of the chick, in pure culture, lose their ability to produce hyaline sheath substance, developing as an undifferentiated epithelium-like tissue quite different from normal cartilage (Fischer, 35). Cartilage from embryonic bone, freed from perichondrium, does not grow at all (34). But when a pure culture of heart fibroblasts and a pure culture of epitheloid tissue derived from cartilage are grown side by side in the same medium, at the point of contact a new tissue, actively growing typical cartilage, arises and can be carried on indefinitely (34). This is then no longer a "tissue culture" in the strict sense of being a pure-line culture of a single type of cell, and cartilage is not, strictly speaking, a "tissue." This point of view has been presented by Parker, Fischer and Parker, Fischer, Fell, and others, and shows the absurdities that result from trying to draw too sharp a line between what is and what is not a tissue culture.

Work with root cultures is being continued in an effort to simplify the method and make it more reliable, and these results are being applied to practical problems of physiology and pathology. One of the unfortunate features of the method is the rigid control necessary if satisfactory results are to be obtained. Such isolated plant organs are extraordinarily sensitive. Many of the important cultural variables have been studied in some detail (temperature, pH, etc.), but others have apparently even eluded identification. The sensitiveness of such roots is shown, for example, by the fact that increasing the total salt concentration of the solution used from 2.6 millimols (the optimal concentration) to 10 millimols, both extremely low absolute concentrations, will reduce the growth obtained by about 60% (unpublished results). A change of maintained temperature from 27° to 30°, a difference of only 3° but across a critical range, will reduce the growth rate of wheat root-tips from the normal average value of about 5 mm. per day to almost nil (125). Decreasing the Fe-ion concentration from .003 milliequivalents to .002 milliequivalents will decrease the tissue yield by 50% (126). The roots are even more sensitive to such variants as the cystein-HCl studied by Gautheret (44). Moreover, there appears to be marked specificity as regards carbohydrate re-

quirements, since tomato requires sucrose, being unable to utilize dextrose under the conditions studied, while wheat gives excellent results with dextrose. Efforts to increase the phosphate concentration so as to make possible buffering of the solution have regularly proved fatal. Failure to control properly some one or more of these and possibly unknown factors is doubtless responsible for the checks encountered by other workers, and it can not be overemphasized that the nutrient formulae, cultural conditions, etc., as published, can with certainty be applied *only* to the species of plant described.

As Haberlandt foresaw (46), the cultivation of excised plant tissues has thrown many interesting lights on the behavior of certain groups of cells even though single cells have not been successfully isolated. The *apparent* dependence of chlorophyll-free tissues on moderate illumination, as reported by Robbins and Maneval (103), White (125), and Felber-Pisk (28), but denied by Malyschev (82), is interesting and will require further verification. The necessary character of iron, usually thought of as merely a catalyst for chlorophyll formation, for the proper maintenance of a chlorophyll-free tissue (126), is likewise interesting, but quite in keeping with Warburg's concept of the relation of iron to respiration in general (121). The fact that tomato roots are able to utilize sucrose under the conditions studied, but can not utilize dextrose, indicates that either sucrose, contrary to earlier opinion, can enter the cells as such and is there utilized directly or broken down to materials other than dextrose and levulose, or else that roots secrete into the medium some hydrolyzing agent giving rise to materials other than these hexoses. The proved unlimited capacity for growth of such cultures (130) obviously sets aside the concept of indispensable and *specific* correlation hormones, since the only possible source of such hormones has been the thermo-stable, apparently non-protein (Osborne and Wakeman, 92) filtrate of a plant (yeast) taxonomically and physiologically widely separated from the cultured tissue. These and many other interesting facts have been brought to light by the studies made to date, and give some indication of the potential value of this method of approach. It is to be noted that every factor in the environment, with the exception of the yeast extract, is of known character and can be controlled and varied at will, so that all sorts of problems of nutrition

and of other phases of physiological behavior can be approached in this way. The method represents a powerful new tool for such studies. Unfortunately, perhaps because of the sensitiveness of such cultures and the need for rigorous control if results satisfactory for physiological studies are to be obtained, the method has not as yet taken a prominent place in the plant physiologists' armamentarium. It has received somewhat more attention from the pathologists and has been used as a means of maintaining pure-line cultures of obligate parasites (viruses, 131), and in the study of nodule-forming bacteria (Lewis and McCoy, 77). The latter work gave unsatisfactory results, probably due to faulty technique. Riker and Berge have suggested the use of the method in studies of the crown gall organisms (99). It is to be hoped that, with the method itself firmly established, its application will be extended to other fields.

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